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Original Article

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Failure of artemether-lumefantrine therapy in travellers returning to Belgium with *Plasmodium falciparum* malaria: an observational case series with genomic analysis

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Abstract

Background: Failure of artemisinin-based combination therapy is increasingly reported in patients with *Plasmodium falciparum* malaria in sub-Saharan Africa. We aimed to describe the clinical and genomic characteristics of recent cases of *P. falciparum* malaria failing artemether-lumefantrine in Belgium.

Methods: Travel-related cases of malaria confirmed at the national reference laboratory of the Institute of Tropical Medicine, Antwerp, Belgium, were reviewed. All cases for which attending clinicians reported persistence (beyond Day 3 post-treatment initiation, i.e. early failure) or recrudescence (from Day 7 to 42, i.e. late failure) of *P. falciparum* parasites despite adequate drug intake were analysed. Both initial and persistent/recurrent samples were submitted to next generation sequencing to investigate resistance-conferring mutations.

Results: From July 2022 to June 2023, eight *P. falciparum* cases of failure with artemether-lumefantrine therapy were reported (early failure = 1; late failure = 7). All travellers were returning from sub-Saharan Africa, most (6/8) after a trip to visit friends and relatives. *PfKelch13 (PF3D7_1343700)* mutations associated with resistance to artemisinin were found in two travellers returning from East Africa, including the validated marker R561H in the patient with early failure and the candidate marker A675V in a patient with late failure. Additional mutations were detected that could contribute to decreased susceptibility to artemisinin in another three cases, lumefantrine in six cases and proguanil in all eight participants. Various regimens were used to treat the persistent/recrudescent cases, with favourable outcome.

Conclusion: Within a 12-month period, we investigated eight travellers returning from sub-Saharan Africa with *P. falciparum* malaria and in whom artemether-lumefantrine failure was documented. Mutations conferring resistance

to antimalarials were found in all analysed blood samples, especially against lumefantrine and proguanil, but also artemisinin. There is a pressing need for systematic genomic surveillance of resistance to antimalarials in international travellers with *P. falciparum* malaria, especially those experiencing treatment failure.

Key words: Malaria genomic surveillance, travel, artemisinin-based combination therapy, failure, drug resistance

Introduction

In Europe, malaria remains among the leading causes of travel-related morbidity and mortality,[1,2] with 4856 cases reported in 2021 by the European Centre for Disease Prevention and Control (ECDC),[3] of which 85% were due to *Plasmodium falciparum* and <1% were autochthonous. This number was still much lower than those reported before the COVID-19 pandemic (about 8000 cases yearly since 2016), but reflected already a sharp increase compared with the 2480 cases notified in 2020.

Oral artemisinin-based combination therapies (ACTs) are the first-line drugs recommended by the World Health Organization (WHO) for the treatment of uncomplicated malaria, or as adjunctive therapy for severe malaria after the initial administration of intravenous artesunate.[4] ACTs combine a highly effective short-acting artemisinin derivative (i.e. artemether, artesunate or artenimol), which rapidly reduces the parasite biomass, with a longer-acting antimalarial partner drug (e.g. lumefantrine and piperaquine for ACT used in Europe), aimed to eliminate the remaining parasites and protect against resistance to artemisinin. The clinical efficacy of ACTs against *P. falciparum* is under scrutiny in endemic countries, as resistance to artemisinin and to various partner drugs has already spread in Southeast Asia[5] and recently independently emerged in Africa.[6] Treatment failure is defined as the inability to clear malarial parasitemia or prevent recrudescence after administration of a full antimalarial treatment, regardless of clinical symptoms. In the non-endemic setting, where reinfection is unlikely, many factors can however contribute to treatment failure, including incorrect dosage, non-compliance, suboptimal absorption, overweight, poor drug quality, drug interactions, high initial parasitemia and parasite resistance to the given treatment.[7,8] A distinction is made between early and late treatment failure. Early treatment failure is defined as persistence of parasites in blood beyond 3 days after treatment initiation (i.e. delayed clearance), which can reveal decreased susceptibility to artemisinin. Late treatment failure is defined as parasites reappearing 7–42 days after treatment initiation and may be related to resistance to aretemisinin and/or the partner drug.[7]

Most of the known mechanisms by which *P. falciparum* develops resistance to antimalarial drugs are related to changes in the parasite genome, such as single nucleotide polymorphisms or gene amplifications. Reduced susceptibility to artemisinin is primarily determined by mutations in the propeller region of *Plasmodium falciparum Kelch 13* (*PfK13*) gene.[9] *PfK13* mutations are now widespread in the Great Mekong subregion, but these mutations have been uncommon in Africa.[10–12] However, recent reports have demonstrated the presence of *PfK13* mutations associated with delayed clearance, first in Rwanda[13] and later in neighbouring countries.[14] Mutations in the propeller domain of the *Plasmodium falciparum coronin* (*Pfcoronin*) have also been associated with reduced susceptibility to artemisinin in *P. falciparum* African strains in culture.[15] Decreased activity to lumefantrine is mainly associated with *Plasmodium falciparum chloroquine resistance transporter* (*Pfcrt*) gene and *Plasmodium falciparum multidrug resistance protein 1* (*Pfmdr1*) gene genes and to piperaquine with *Plasmodium falciparum plasmepsin* (*Pfplasmepsin*) 2–3 copy number.[16]

In 2019, we reported on six cases of late treatment failure of *P. falciparum* malaria, observed from 2014 to 2017 at the Institute of Tropical Medicine (ITM), Antwerp, after treatment with either artemether-lumefantrine (AL) or artenimol-piperaquine (AP).[17] All recrudescences occurred in non-immune Caucasian travellers and five of them had severe malaria and/or hyperparasitemia at initial presentation (defined as blood stage parasite density above 200 000 asexual parasites/μL of blood). Genetic analysis did not support parasitological resistance to ACT, suggesting that failure was related to inadequacy of partner drug dosage or of treatment duration in non-immune populations.[8] Similar observations were made in contemporary series from Sweden[18] and UK.[19]

In this study, we report on the clinical and genomic details of new cases of ACT failure that were observed in several Belgian travel clinics from mid-2022 to mid-2023.

Methods

Diagnosis and epidemiological surveillance of malaria in Belgium

Malaria cases are diagnosed in clinical laboratories of Belgian hospitals and travel clinics using conventional light microscopy of Giemsa-stained thin and thick blood smears, immunochromatographic rapid diagnostic tests and more recently also loop-mediated isothermal amplification (LAMP) tests. Most Belgian laboratories send malaria positive samples (on a voluntary basis) to the clinical laboratory of ITM which hosts the reference laboratory for malaria in Belgium. A recent internal survey estimated that ~80% of the malaria cases diagnosed in Belgium are currently captured. All samples diagnosed with whatever method are subjected for confirmation to molecular analysis with a polymerase chain reaction (PCR)-based method detecting the four most common *Plasmodium* species.[20] An additional PCR targeting *Plasmodium knowlesi* is performed in any malaria case not confirmed by the quadruplex PCR.

Annually, the ITM declares the numbers and species of confirmed malaria cases to the Federal Public Health and Veterinary Institute (Sciensano), which reports aggregated national data on malaria cases to the ECDC. The notification of malaria is however not mandatory in Belgium, except when autochthonous cases are suspected.[21]

Treatment and follow-up

Belgian recommendations for malaria management are based on the WHO guidelines.[4] Whenever malaria is diagnosed, patients

are systematically assessed for clinical and laboratory features of severity according to WHO criteria.[4,22] Severe malaria is treated with intravenous artesunate (for at least one day) and supportive care, followed by an oral course of AL (Riamet®, Novartis; six doses for 3 days, given as four tablets/dose for patients >12 years) when the patient is stabilized and able to swallow. Uncomplicated malaria is directly treated, either in the ambulatory setting or under hospital supervision, with AL as described above. Of note, Riamet® is the only ACT commercially available in Belgium since 2019, but some patients may be treated with AP (Eurartesim®, Alfasigma) from previous stock or imported from abroad. Atovaquone-proguanil (APG) (Malarone®, GlaxoSmithKline or various generic formulations) can be used as second-line therapy for uncomplicated malaria in Belgium.

Once treatment is started (Day 0), blood smears are usually taken every 24 hours in hospitalized patients to monitor the parasitological response, or may be performed during a follow-up visit at Day 3 to 5 in outpatients. For severe cases, an additional visit is systematically scheduled after 2 weeks to detect any late hemolysis related to intravenous artesunate and/or oral ACT.[23,24] Patients are informed about the risk of malaria recrudescence and asked to immediately attend a specialized clinic to repeat malaria testing in case fever recurs within 6 weeks.

Management of treatment failure

Based on the recent experience of recrudescent malaria,[17] infectious diseases specialists in Belgium have been alerted to rapidly contact the reference ITM clinical or laboratory team in case of an unusual course, such as parasite positivity persisting beyond 3 days after treatment initiation, or parasitemia re-occurring up to 6 weeks after the initial episode (if there has been no new exposure). After a thorough clinical review, if treatment failure was not explained by an evident lack of compliance, demographic and clinical data were collected, and all available serial samples of initial and persistent/recurrent episodes were sent to ITM. At the request of clinicians, *ad hoc* genetic analyses were performed on the paired samples, in search of resistance-conferring mutations to artemisinin and/or the partner drug, for surveillance purposes. As those results were not immediately available, a case-by-case discussion between ITM experts and the treating physician was initiated to select the best therapy for persistent/recurrent cases. Either a new ACT course or a treatment with atovaquone/proguanil could be proposed, as there is no international recommendation on the optimal way to treat such treatment failures in the non-endemic setting. Close follow-up of recovery and documentation of cure were strongly advised for all failing cases, and those data were also collected.

Genomic analysis of resistance markers in treatment failure cases

Serial whole blood samples of failing cases (at initial presentation and at persistence/recrudescence) were sent to the Malariology Unit of ITM. DNA was extracted using QIAamp DNA Blood Mini Kit QIAGEN GmbH, Germany) from 200 μL of blood and resuspended in 200 μL of water. Parasite genetic markers associated with ACT resistance were investigated using an in-house developed amplicon targeted next generation sequencing (NGS) assay (AmpliSeq).[22] Details on the laboratory methods and the bioinformatic analysis performed have been previously published.[25,26] This NGS assay has been adapted for African strains of *P. falciparum* and covers 14 genes, including *PfK13*, *Plasmodium falciparum dihydrofolate reductase* (*Pfdhfr*), *Pfmdr1, Pfcrt, Plasmodium falciparum dihydropteroate synthetase* (*Pfdhps*), *Pfcoronin* and *Plasmodium falciparum Apicoplast Ribosomal Protein S10* (*Pfarps10*), which have different levels of association to ACT resistance. As the gene *Plasmodium falciparum cytochrome b* (*PfCytB*), associated with atovaquone resistance, is not included in the AmpliSeq panel, a targeted PCR was performed[27] in batch in November 2023 and amplified product was shipped for Sanger sequencing to Genewiz (Takeley, UK).

Ethics

At the ITM and two other academic institutions (University Hospital of Antwerp and Ghent), an optout strategy has been put in place regarding use of deidentified clinical data and leftover of samples for epidemiological surveillance of travel-related infections, with the clearance of the respective Institutional Review Board/Ethic Committee. For both patients included in the other two hospitals without such a policy, individual written or verbal consent was obtained.

Results

Trends of travel-related malaria cases diagnosed at the ITM reference laboratory

After a sharp decrease in 2020, the number of malaria cases diagnosed and/or confirmed at the ITM reference laboratory rebounded up to 499 in 2022 (Figure 1), the highest annual incidence in a decade (4.3 cases/100 000 inhabitants). Another 212 cases were still diagnosed in 2023 (until end of June). *P. falciparum* was the largely predominant species (>85%). The traveller characteristics and countries of acquisition were similar to what was published in detail in 2019.[17]

After a 4-year period (2018–2021) during which no case of ACT failure was reported to the ITM reference laboratory, eight cases of persistent or recurrent fever and *P. falciparum* parasitemia were notified from July 2022 to June 2023 (period during which 430 *P. falciparum* cases were diagnosed, corresponding to a failure rate of 1.9%). Of note, two additional cases of treatment failure reported to ITM were not included in this study because they were obviously related to incomplete drug intake.

Characteristics and outcome of the cases of ACT failure

The epidemiological, clinical and laboratory characteristics of both initial and persistent/recurrent malaria episodes are reported in Table 1. All eight patients returned from African countries and none had taken adequate chemoprevention. Six were travellers visiting friends and relatives (VFR). All failing cases had been initially treated with AL (including one with transient impaired consciousness). Treatment intake had been fully supervised in hospital for seven of them and appropriate

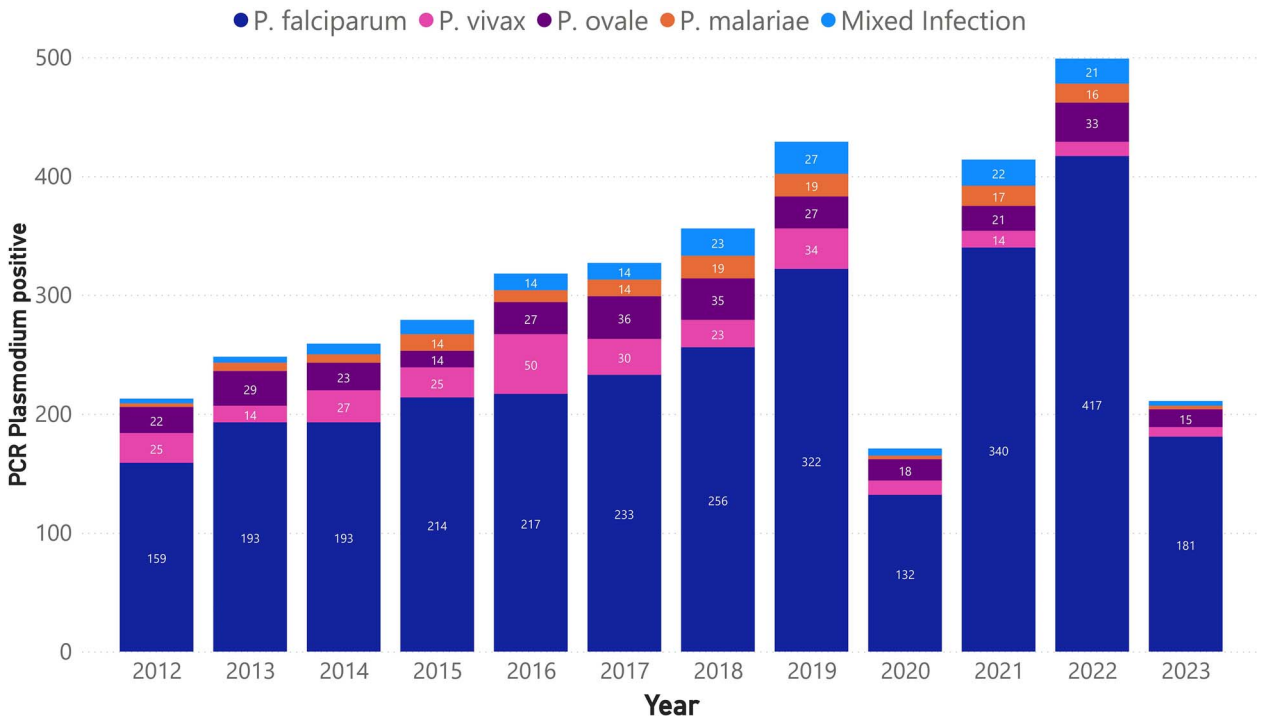


Figure 1 Number of malaria cases, by species, diagnosed at the ITM reference laboratory from 2012 to June 2023

compliance had been thoroughly reviewed for the remaining case treated as outpatient.

One case (Patient 4, returning from Burundi) fulfilled the criteria of early treatment failure with persistent fever and parasitemia beyond Day 3, up to Day 8. He received APG at that moment and recovered quickly. All other seven patients presented with late treatment failure. All recrudescent episodes occurred between 15 and 29 days after initiation of treatment. Duration of fever before the diagnosis of recrudescence ranged from 2 to 6 days. The recrudescent cases were treated with either AL ($n=4$), AP ($n=2$) or APG ($n=1$). Three of them got first intravenous artesunate, one because of severe malaria (impaired consciousness) at recrudescence and the remaining two while waiting for expert's advice. All recovered and none had any additional recrudescence.

Genomic analysis of the cases of ACT failure

As shown in Figure 2, AmpliSeq sequencing detected the mutations A675V and R651H in the *PfK13* in two patients. The R651H mutation is a WHO validated resistance marker for ART[28] and was detected in the only patient with early treatment failure (Patient 4). The A675V mutation is a WHO candidate resistance marker for artemisinin,[28] which was observed in a patient with late treatment failure (Patient 1, returning from Uganda). We also detected S183G in the propeller domain of the *Pfcoronin* gene in three patients (including the one with A675V), a mutation worth reporting as it does not confer resistance by itself *in vitro* but is often intertwined with other artemisinin resistance mutations.[15,29] An additional mutation in *Pfcoronin*, P76S, was detected in one patient. Although it did not appear to be predictive of *in vitro* susceptibility to artemisinin or partner drugs,[30] this mutation was associated with clinical treatment failure in a recent Israeli case series.[31] The combination of wild types at *Pfcrt* (K76T) and *Pfmdr1* (N86Y and D1246Y) with the Y184F mutation at *Pfmdr1*, which has been associated with higher tolerance to lumefantrine *in vitro* and *in vivo*,[32] was detected in six failing patients.

Of note, all patients were also infected with double ($n=1$) or triple ($n=7$) *Pfdhfr*-mutants (N51I, C59R and S108N), widely documented to be associated with proguanil resistance, the partner drug of the non-ACT combination APG.[33] In contrast, the A803C mutation in *PfCytB* validated for atovaquone resistance was absent in all samples. In fact, no polymorphisms were detected in the *PfCytB* gene, confirming that all patients were carrying the wild type parasite.[33] Additional mutations, detected with the used AmpliSeq assay and for which clinical relevance is still unclear, can be found in the Supplementary Table S1 for non-synonymous mutations in *PfK13* and in Supplementary Table S2 for all genes included in the AmpliSeq panel.

Discussion

We report on the detailed investigation of eight cases of *P. falciparum* malaria, diagnosed in Belgium from July 2022 to June 2023 after a stay in sub-Saharan Africa, and who failed AL treatment despite adequate intake. Different combinations of mutations conferring resistance to artemisinin and/or relevant partner drugs were observed in all these failing patients, including the first-time detection of a *PfK13* validated resistance marker (R561H) in a traveller with early treatment failure after a stay in Burundi. Mutations associated with decreased susceptibility to lumefantrine were detected in most cases.

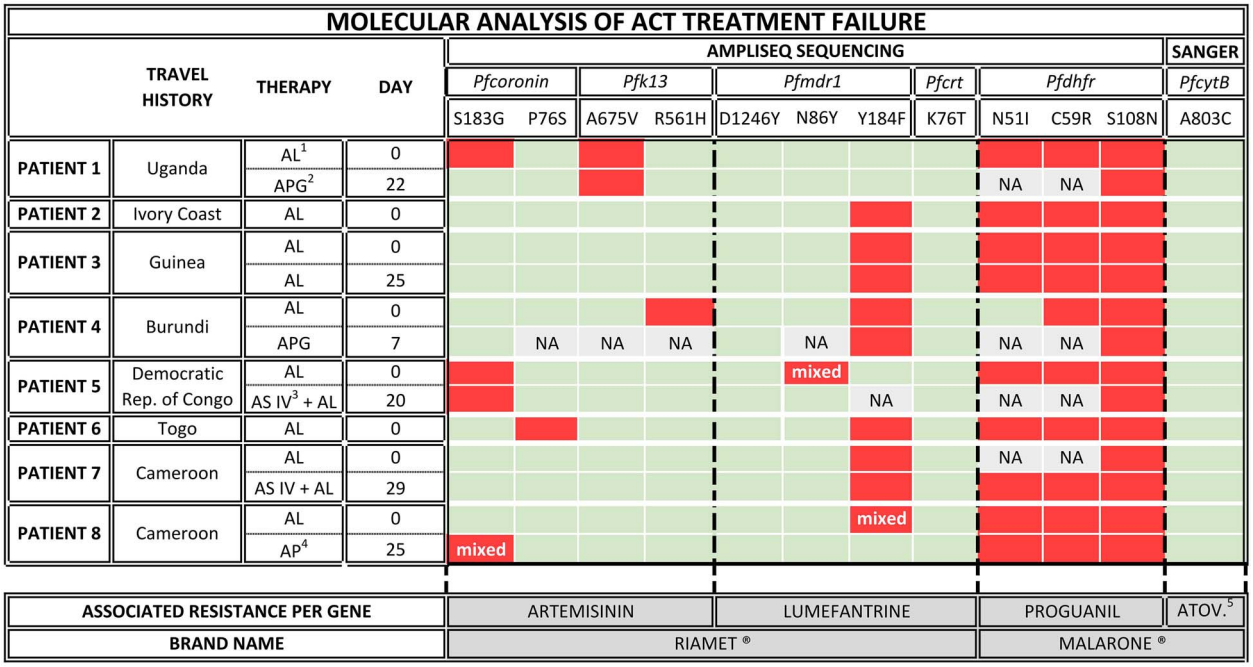
Table 1 Epidemiological, clinical and laboratory features of cases with treatment failure (2022–2023) at ITM

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
General characteristics								
Gender	Male	Male	Male	Male	Female	Male	Male	Male
Age (years)	70	25	21	33	46	35	51	19
Weight (kg)	78	85	68	80	103		83	70
Country of birth	Belgium	Ivory Coast	Guinea	Burundi	DR Congo	Belgium	Cameroon	Cameroon
Travel destination	Uganda	Ivory Coast	Guinea	Burundi	DR Congo	Togo	Cameroon	Cameroon
Travel date	Mar–Jun 2022	Jul 2022	Aug 2022	Jul–Sep 2022	Sep–Oct 2022	Dec 2022	Mar–Apr 2023	Mar–Apr2023
Travel duration	3 m	3 w	2 w	2 m	1 m	3 d	1 m	1 m
Chemoprophylaxis	Doxycycline (incorrect)	No	No	No	No	No	No	No
First episode (Day 0)								
Days with fever before diagnosis	Not available	3	7	2	11	4	2	9
Parasitemia at diagnosis (/μL)	69 557	205 200	49 422	24 904	3708	33 680	48 660	38 371
Criteria of severe malaria	Impaired consciousness	None	None	None	None	None	None	None
Treatment	AL	AL	AL	AL	AL	AL	AL	AL
Duration of treatment (days)	3	3	3	3	3	3	3	3
Treatment setting	Hospital	Hospital	Hospital	Hospital	Ambulatory	Hospital	Hospital	Hospital
Parasitemia at Day 3	Undetectable	Undetectable	NP	NP	NP	Undetectable	Undetectable	NP
Follow-up negative parasitemia at	None	D12	D5		D9	D9	None	D5
Recurrent/persistent episode								
Days after first diagnosis	22	23	25	7	18	15	29	25
Days with fever before diagnosis	No fever	1	3	Persisting fever	3	2	6	6
Parasitemia at diagnosis	4319	17 600	40 066	25	61 141	29 991	4138	5825
Criteria of severe malaria	None	Impaired consciousness	None	None	None	None	None	None
Treatment	APG	Artesunate IV (1d) followed by AP	AL	APG	AL	Artesunate IV (1d) followed by AL	Artesunate IV (1d) followed by AL	AP
Duration of treatment (days)	3	4	3	3	3	4	4	3
Treatment setting	Ambulatory	Hospital	Hospital	Ambulatory	Hospital	Hospital	Hospital	Hospital
Follow-up negative parasitemia (D0 = start date second treatment) at	D4	D3	NP	NP	D3, D11	D3, D8, D30	D13, D30	D2, D27

Note: AL, artemether-lumefantrine; AP, artenimol-piperaquine; APG, atovaquone-proguanil; NP, not performed; D, day(s); w, weeks; m, month(s).

A substantial post-lockdown re-increase in the malaria caseload has also been observed in other Western countries (data available up to 2021),[3,34,35] and some evidence suggests that the proportion of severe malaria has increased.[35,36] It remains unclear so far whether it is related to a 'transient' catchup of VFR travel, following the COVID-19 restrictions, or if this upward trend will be sustained, as malaria control stagnates in sub-Saharan Africa.[37,38] It highlights again the importance for the medical community at large to maintain preventive messages and interventions, with a specific focus to the VFR population, at highest risk of malaria in all recent studies.[17,39]

In this new cases series of ACT failure, there were some striking differences compared with our previous cohort.[17] First, the eight failing cases were observed within a 12-month period, after 4 years with no notification, and while less than two cases per year came to our attention in average from 2014 to 2017. This abrupt disproportionate peak of cases is still difficult to interpret in a longer-term perspective, but suggests a true increase in incidence. A similar trend in the proportion of AL failures has also been observed in Israel.[31] Second, most study patients were VFR travellers, and all but one presented with an initial parasitemia below 2% of red blood cells, in sharp contrast with the failing patients evaluated between 2014 and 2017 who were mostly non-immune travellers often presenting with high parasite densities. In addition, one patient had an early treatment failure (persistent parasitemia up to 8 days after



Footnotes: [1] Artemether-Lumefantrine combination therapy (Riamet); [2] Atovaquone-Proguanil (Malarone); [3] Intravenous Artesunate; [4] Artenimol-piperaquine combination therapy (Eurartesim); [5] Atovaquone

TABLE 2. LEGEND

: absence of mutation (wild type)
: presence of mutation (each patient having a color-ID)
NA : missing data after sequencing
mixed : both wild type and mutant detected (mixed infection)

Figure 2 Description of mutations detected among the *P. falciparum* strains during the initial and persistent/recrudescent malaria episodes, by AmpliSeq new genome sequencing, with focus on mutations likely associated with reduced susceptibility to antimalarials used in Belgium

treatment initiation), an occurrence we never observed before. Finally, all recent cases presented with mutations associated with decreased susceptibility to artemisinin and/or partner drugs in the ACT, while in the previous series, none of the tested mutations was present and hence, could explain treatment failure. Of note, after the discussion with ITM experts, varied regimens were administered to treat the persistent/recrudescent cases, including AL, AP or APG, sometimes preceded by intravenous artesunate. This highlights the important area of uncertainty regarding the current clinical management of such failing cases.

The presence of different sets of resistance-conferring mutations in all analysed failing cases is worrisome for the future care of travellers with (*P. falciparum*) malaria. The *PfK13* R561H mutation, associated with artemisinin resistance in southeast Asia[10,28] and detected first in Rwanda in 2014,[13] is expanding throughout the wider Great Lakes region with recent reports from Uganda and Tanzania.[14] The observation that this validated resistance marker was carried by a traveller infected in Burundi represents the first report linked to this country. The *PfK13* A675V mutation, associated with delayed clearance, was previously reported in Rwanda and multiple sites across Uganda.[40] To our knowledge, this is the second published report of a traveller returning from East Africa carrying this candidate resistance marker.[41] This first case exhibited delayed clearance under artesunate, while our patient presented with late failure. Another *P. falciparum* case of early ACT failure after a stay in Tanzania in 2018 has been reported, but no mutation could be found in the propeller region of *PfK13*.[42] The presence of mutations in the *Pfcoronin* gene in four patients (three with S183G; one with P76S) is another source of concern for artemisinin efficacy. In a case series from Israel,[31] while no mutations in the *PfK13* gene were identified, two mutations in the *Pfcoronin* gene were observed (P76S and S183G) in some patients, but only P76S was more frequently detected in failing patients compared with treatment responders. In addition, the genomic pattern of most of our cases was also indicative of decreased activity of lumefantrine, which could have contributed to late treatment failure in several patients. Indeed, with the replacement of chloroquine by ACTs as first-line treatment in Africa, the prevalence of mutations associated with chloroquine resistance (*Pfcrt* K76T and *Pfmdr1* N86Y and D1246Y) is decreasing in several regions.[43–45] The reversion to wild type is associated with lower susceptibility to lumefantrine in presence of the *Pfmdr1* Y184F mutation.[46] Finally the presence of cumulative mutations in the *Pfdhfr* gene, associated with proguanil resistance, may, in turn, jeopardize the preventive and curative activity of the combination APG. However, in our study, no mutation was detected that confers resistance to atovaquone. Overall, the detection of some key mutations may suggest that resistance (at least partial) could have contributed to treatment failure. However, four cases who initially failed AL cured uneventfully with a second course of

the same drug, underlining the fact that other, host or pathogen, genetic or non-genetic, factors may also have played a role in the initial non-response. Also, the efficacy of APG in two cases despite resistance markers against proguanil may suggest that this latter drug mainly acts synergistically rather than directly on the parasites.

Sick travellers returning from various tropical countries serve as sentinels for many infectious diseases that could go unrecognized where diagnostic facilities are scarce. A systematic and multicentric genomic surveillance of resistance markers in international travellers with malaria could be instrumental in detecting emergence of drug resistance globally and timely. At least, reactive molecular investigation of failing cases should be progressively implemented in reference travel clinics.

There are some limitations in this study. We do not provide accurate incidence data of treatment failure in the total number of *P. falciparum* malaria cases seen in Belgium. Indeed, the sending of malaria samples to the reference laboratory of ITM is voluntary-based, implying that national coverage is not complete. Similarly, the genomic analysis of resistance markers in patients with persistent/recrudescent malaria is performed on an *ad hoc* basis, whenever ITM physicians are alerted by colleagues concerned about abnormal evolution during or after treatment. Therefore, the genomic picture provided here may also be incomplete. It must be underlined however that awareness on the risk of ACT failure has increased among Belgian clinicians following the publication of our previous report,[17] suggesting that few cases were likely missed. Finally, as study inclusion was made at the recrudescent episode, no measurement of drug concentration could be performed during the initial episode. There are also several strengths. Clinical data were systematically collected for each successive persistent/recrudescent case soon after inclusion, and treatment adherence and potential drug interactions could be immediately and thoroughly reassessed, leading to the exclusion of two non-compliant cases (not reported here). Genomic analysis of resistant markers was reactively obtained within a rather short timeframe and, even if it did not immediately impact the clinical management, raised new awareness that parasite tolerance could also contribute to treatment failure, with need of careful monitoring. Finally, the use of amplicon targeting NGS (AmpliSeq) has enabled the investigation of a large number of not only validated but also potential and contributing mutations associated with drug resistance, which would be tremendously laborious to examine by PCR-based methods.

Conclusion

The rising number of cases of ACT failure in travellers returning from sub-Saharan Africa with genomic evidence of resistance-conferring mutations is worrisome. Emergence of markers of resistance against artemisinin derivatives in some collected samples is all but surprising however, considering the recent epidemiological trends in sub-Saharan Africa. Clinicians should be aware of this trend, and adequately inform malaria patients about the non-negligible risk of treatment failure. International initiatives to setup a global monitoring of resistance of *P. falciparum* malaria should be strongly supported. Research to improve the clinical management of persistent and recrudescent *P. falciparum* infections in travellers should get the highest priority.

Supplementary data

Supplementary data are available at *JTM* online.

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Authors' contributions

Conceptualization and design of the study: J.P., E.B., D.K., M.V.E., M.M., A.R.U.

Collection and curation of clinical data: J.P., E.B., E.F., U.M., A.B., J.M., C.M., J.Cox, D.K.

Laboratory analysis: P.G., J.V., J.Coppens, M.V.E., M.M., A.R.U.

Data analysis: J.P., E.B., M.V.E., M.M., A.R.U.

Drafting, reviewing and editing of the manuscript: all authors.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Conflict of interest: None declared.

Data availability

The clinical data cannot be shared publicly in order to protect to the privacy of individuals associated with this study. Aggregate data could be shared on request to the corresponding author. Full genomic data are available as Supplementary Material.

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